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Remarkable enhancement of bacterial inactivation in wastewater through promotion of solar photo-Fenton at near-neutral pH by natural organic acids

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ABSTRACT

In this work, the near-neutral photo-Fenton process, as a means of wastewater disinfection, was enhanced by the addition of Organic Acids (OAs), namely citric, ascorbic, tartaric and caffeic acid. The addition of OAs exhibited significant bacterial inactivation enhancement, compared to the classic photo-Fenton systems (Fe^{2+}/H_2O_2/solar and Fe^{3+}/H_2O_2/solar). The improved disinfection performance was not attributed to pH variations by the addition of OAs, but to the increase of the initial dissolved iron in the system, facilitating the Fe^{3+}/Fe^{2+} turnover in the catalytic photo-Fenton reaction and consequently, the hydroxyl radicals production. For citric and tartaric acid, increased photo-activity of the complexes was associated with their high capability to complex Fe^{3+} and to promote ligand-to-metal charge transfer (LMCT), which is of key importance to feed Fe^{2+} to the Fenton process. On the other hand, for ascorbic and caffeic acid, the acceleration of the homogeneous Fenton reaction was attributed to both their complexing and reductive properties of the acids. Both effects contributed to the enhancement of the *E. coli* inactivation. Moreover, the addition of OAs extracted from natural origin (lime, orange, coffee) showed a notable enhancement of the bacterial inactivation, attributed to the presence of the previously tested organic acids as key constituents of the selected natural products. Although the addition of synthetic or natural OAs into the system increased COD and DOC, bacterial inactivation and organic matter self-degradation was simultaneously and efficiently realized, making it a green and sustainable improvement method.

1. Introduction

Advanced Oxidation Processes (AOPs) have shown to be a promising option for disinfection process [1–3] and for the degradation of organic pollutants present in wastewaters [4–8]. The efficiency of these processes is based on the formation of reactive species, especially hydroxyl radicals ($HO^{\bullet}$), a highly reactive and non-selective species, able to oxidize organic molecules. Among the AOPs, the photo-Fenton process has shown to be highly efficient for the mineralization of organic pollutants and bacterial inactivation making of this process an interesting option for wastewaters treatments [9–11].

At acidic pH, the Fenton efficiency, which is optimal at pH around 3, is attributed to the fast formation of $HO^{\bullet}$ through the decomposition of H_2O_2 by ferrous ions (eq. (1)). However, this process is limited by the very low constant rate of regeneration of Fe^{2+} from Fe^{3+}, via the reduction by H_2O_2. This limitation is minimized in the photo-Fenton process in acidic conditions by the interaction of light radiation (eq. (2)).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^{\bullet} \quad (1)$$

$$Fe(H_2O)_5(OH)^{2+} + h\nu \rightarrow Fe^{2+} + HO^{\bullet} \quad (2)$$

These conditions however do not apply at the pH values usually found in wastewaters. At neutral pH, Fe^{3+} is quickly transformed to insoluble Fe oxides, which have limited efficiency as photocatalysts, when compared to the homogeneous reactions by soluble iron forms. Therefore, research should be focused to solutions that enhance the operational capabilities at near-neutral pH.

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Previous investigations reported the effective production of Fe^{2+} from ferric organo-complexes by LMCT (Eq. (3)) [12–14]. During this step, an oxidized ligand ($R^{\bullet}$) is produced, which in presence of dissolved oxygen undergoes into a multiple step reaction that may involve several intermediates ending up in the production ROS (Eq. (4)) that could also contribute in the process. The formation of Fe^{3+}-complexes with organic chelates increases the soluble iron in the system and the process can take place at near-neutral pH [15]. Additionally, organic ligands make more interesting the use of solar radiation in the system by increasing the absorbance spectra of the visible region turning into the Solar Photo-Fenton process.

$$[Fe(RCOO)]^{2+} + h\nu \rightarrow Fe^{2+} + R^{\bullet} + CO_2 \quad (3)$$

$$R^{\bullet} + O_2 \rightarrow O_2^{\bullet -} + CO_2 \quad (4)$$

However, many of the tested organic molecules as ligands (EDTA, nitriloacetic acid among others) [16–18] are not commonly found in the natural environment representing a risk for human health making the process unviable for water treatment [19]. The search for not harmful chelates agents for iron complexes becomes a new challenge. For this reason, the structural isomer of EDTA, EDDS, was considered instead, being an environmentally safe alternative [18,20,21]. Furthermore, the use of soluble bio-based substances (SBOs) was assessed [22,23], as an interesting method to promote photo-Fenton. Another possible solution to this situation is the use of organic compounds able to produce ferric complexes that can be found in natural conditions. Among the organic compounds that could act as ligands, organic acids are highlighted given their possible deprotonation at pH values around 7.0 and then they easily complexes Fe^{3+} ions [14,19].

Low weight organic acids have been tested in the photo-Fenton process showing the capacity to produce reactive species given their photo-active properties and their ability to increase the soluble iron in the system [14,15,24,25]. For instance, previous investigations proposed citric acid as a good alternative to promote ferric complexes for the photo-Fenton process and reach the degradation of organic pollutants [15,26] or to enhance the inactivation of *E. coli* by photo Fenton process at near-neutral pH [27]. Moreover, citric acid represents a main constitute of acidic fruits as lemon, lime, orange, among others [28,29]. Other organic acids highly contained in fruits are the tartaric acid [30] ascorbic acid [30] and caffeic acid [31].

Similarly to citric acid, tartaric acid (TA) is a short molecular weight organic acid with multiple carboxylic acid and OH groups in α position. Some authors have mentioned the complexation of ferric ions with citric acid [32] that could improve the efficiency of the photo-Fenton process. Also, an enhanced effect by the TA during photo-Fenton process have been previously presented [16,33]. Among the organic acids, ascorbic acid (AA) is one of the most important ones for human dietary given its biological activity attributed to its antioxidant property. Several natural products have this acid as a major component making interesting its evaluation as additive in the solar photo-Fenton system. In addition to the aforementioned acids, caffeic acid (CfA) is a carboxylic acid and a catechol derivate commonly found in coffee beans [34]. Besides the carboxylic groups, phenols as catechol, have also shown a remarkable chelating activity to transitions metallic ions leading to photo-active complexes [35] and the production of hydroxyl radicals [36].

Since in many food-related industries, the generated by-products are often considered as wastes, the incorporation of such natural products as potential sources of the mentioned organic acids became interesting, as they still contain significant amounts of the aforementioned acids.

Therefore, in this investigation, we evaluate the use of natural products as organic ligand sources, which could enhance the solar photo-Fenton process and lead to higher *E. coli* inactivation in wastewater. The effect of the different organic acids was sought to be harnessed and their contribution to the existing photo-Fenton pathways was investigated. Primarily, organic acids (citric, tartaric, ascorbic and caffeic) were assayed, followed by the use of natural products containing the aforementioned organic acids. Finally, the possible mechanism of bacterial inactivation enhancement by OAs is discussed.

2. Materials and methods

2.1. Reagents

Ferrous sulfate heptahydrate ≥99.0%, ferric sulfate hydrate 97%, hydrogen peroxide 30%, citric acid ≥99.5%, L-ascobic acid ≥99.0%, L-(+)-tartaric acid ≥99.5%, caffeic acid ≥98.0%, acetate buffer solution pH 4.65, ferrozine 97% and hydroxylamine hydrochloride 99% were purchased by Sigma Aldrich. The organic acids structures are shown in supplementary material (SM 1). Titanium oxysulfate was supplied by Fluka; chloride acid, sulfuric acid and sodium hydroxide were supplied by Merck. All solutions were prepared prior the experiments using Mili-Q water (18.2 MΩ-cm). Experiments of *E. coli* inactivation were carried out using simulated municipal secondary effluent of synthetic wastewater reported by Muthukumaran et al. [37]. The wastewater characteristics are 39 ppm of chemical oxygen demand (COD), 9.8 ppm of dissolved organic carbon (DOC) and pH 7.5.

2.2. Bacterial preparation, cultivation and handling

E. coli K-12 strain (MG 1655) was provided from "Deutsche Sammlung von Mikroorganismen und Zellkulturen". Subsequent cultivations were carried out for bacterial activation. The inoculation and cleaning procedures for the working bacteria preparation was followed as previously reported [38] to finally obtain a 10^9 CFU/mL working solution.

2.3. Inactivation experiments: light source and process details

The solar photo-Fenton experiments were performed using a lab scale Suntest solar simulator from Hanau, employing a 1500-W, air-cooled Xenon lamp, with effective illumination surface of 560 cm^2 and a fixed intensity of 600 W/m^2. A portion of 0.5% of the emitted photons fall within the 290–320 range (UVB) and 7% in the UVA area (320–400 nm), while after 400 nm the solar spectrum is simulated. The solar simulator is equipped with an uncoated quartz glass light tube and cut-off filters for UVC and IR wavelengths. The *E. coli* inactivation tests were carried out through batch tests with Pyrex glass bottle reactors, with constant stirring with a magnetic bar at 300 rpm. Synthetic wastewater (100 mL) was mixed during 10 min with the organic acid additive (pure organic acid solutions or natural products additives) followed by the addition of 5 ppm (0.09 mM) of Fe^{3+} initial concentration, and subsequent mixing for 10 min, in absence of light. The solution was then spiked with 100 μL of *E. coli* working bacteria solution, leading to an initial bacterial concentration of 10^6 CFU/mL. After further 10 min of stirring in the dark a sample was plated, indicating the bacteria initial concentration (t = 0 min). Finally, hydrogen peroxide was added into the system (25 ppm of initial concentration) and sunlight radiation was immediately provided. Aliquots of 1000 μL from the bulk of the solution were used every time in order to guarantee a representative sample of the mixture. Each experiment was performed at least in duplicate.

2.4. Analytical measurements

2.4.1. Bacterial enumeration

The spread plate method for bacteria quantification was applied, as previously reported [39]. Every dilution was plated in duplicates and at least two consecutive dilutions were plated for each experiment. Less than 5–7% of difference was obtained between the replicates, therefore the standard deviation is not plotted.

2.4.2. Iron and hydrogen peroxide quantification

The total dissolved iron was quantified using the ferrozine method [12] and hydrogen peroxide concentration was determined by the titanium oxysulfate method [27]. The absorbance was measured using a UV-1800 spectrophotometer (Shimadzu, Japan). Experiments were performed at least in duplicates and less than 5% of difference was obtained.

2.4.3. COD and DOC determination

The chemical oxygen demand (COD) analysis was performed by the closed reflux method using a digestion reactor with low and high range vials and a Lange Hach GmbH DR/3900 spectrophotometer. The dissolved organic carbon (DOC) was monitored by catalytic oxidation/NDIR combustion (TOC-VCS/N standard model) using a SHIMADZU TOC 500 equipped with an ASI auto-sampler (Schweiz GmbH, Reinach, Switzerland).

2.5. Natural products extractions

The natural products extractions were obtained using commercially available fruits: orange (*citrus tangelo*), lime (*citrus lime*) and coffee (*coffea arabica*). For the orange and lime fruits, both their juices and aqueous extractions from the peels were tested as enhancements in the solar photo-Fenton experiments. The juice (J) was separated from the peel by handle squeezing. The peels were grinded after drying at 60 °C for 24 h. In order to guarantee the extraction of soluble organic acids the infusion method was applied as a common method for several metabolites in natural products [40,41]. Then, 2 g of dried material was mixed with 40 mL of boiling water for 5 min. The mixture was then centrifuged for 5 min at 5000 rpm. For the case of coffee, both coffee grain composition and its industrial processing has been widely investigate and reported [42–44]. Then, a simulation of coffee processing was carried out by removing the peel from the grain and stirring 1 g of fresh material with 5 mL of distilled water using a stainless steel mixer. The natural product extraction of the coffee was obtained from separating the solution from the mixture by filtering through a gauze pad. All the natural products (juices and extractions) used along the investigation were obtained and prepared just before the experiments and immediately used.

3. Results and discussion

3.1. Photo-Fenton constituents evaluation in absence of organic acids: solar, solar/H_2O_2, Solar/Fe^{3+} and photo-Fenton (Fe^{2+},Fe^{3+})

Before experimentation with addition of organic acids, the photo-Fenton process constituents are hereby presented (Fig. 1). Based on the results, bacterial inactivation was observed with the photo-Fenton process using both Fe^{3+} and Fe^{2+} as precursors. Additionally, a notable inactivation was observed during the Solar/Fe^{3+} system, while no significant contribution was obtained for the sole solar radiation and the solar/H_2O_2 system. These differences in the kinetics are mainly associated to synergistic effects when photo-Fenton process is applied considering the contributions of solar radiation and the reactive species when iron species and hydrogen peroxide are in the system.

As recently reviewed by Giannakis et al. [45,46] the photo-Fenton process in WW is a complex process, with antagonistic and synergistic actions taking place. Among the antagonists, besides the self-scavenging by DOM, one needs to consider the presence of bicarbonates as a $HO^{\bullet}$ radical trap. Light, H_2O_2 and iron play their role in establishing an internal photo-Fenton process. Since the enhancements tried in this manuscript are mainly external, we will not extend in endogenous inactivation events; interested readers can consult Giannakis et al., [45,46] and references therein. Same applies for the DOM-related photochemistry and more specifically the production of ROS from DOM irradiation by UV/vis light. Although DOM is reported to act as a sink of ROS, many studies evidence their production by DOM illumination [47–51]. These ROS, such as singlet oxygen and superoxide radical can subsequently attack microorganisms (or the DOM itself) or reduce Fe^{3+} to Fe^{2+}. For the simplification of the subsequent experimental findings and focusing on the organic acids' effect, the effects analytically presented in the aforementioned review shall not be repeated through the discussion.

3.2. Photo-Fenton enhancement by the addition of organic acids: bacterial inactivation results

Fig. 2 shows the *E. coli* inactivation by the solar photo-Fenton process in the presence of citric (CA), tartaric (TA), ascorbic (AA) and caffeic (CfA) acids and compared to the systems in the absence of organic acids using Fe^{2+} and Fe^{3+} as precursors. Three different molar ratios regarding the initial concentration of iron were tested for each acid (1:0.01; 1:0.05; 1:0.1). In general, a faster *E. coli* inactivation was observed when organic acids were added into the system. In order to get more information and understand the results, the hydrogen peroxide evolution was determined along the processes. All the systems showed higher consumption of H_2O_2 ($\geq$10%) when organic acids were added suggesting an increased Fenton reaction activity (Eq. (1)).

Moreover, although the addition of the organic acids could decrease the pH, they did not promote the inactivation of bacteria by themselves since control experiments using CA, TA, AA and CfA showed no variation of cultivable *E. coli* (SM 2–SM 5). Since the contribution of the acidic medium in bacterial inactivation during the process is negligible, the enhancement of the solar photo-Fenton efficiency could be associated to the following facts:

(i) Organic acids and Fe^{3+} produce highly soluble ferric organo-complexes increasing the amount of dissolved iron in the system. The measurements of total dissolved iron corroborated that in the presence of all the tested organic acids, the initial amount of dissolved iron in the system is significantly larger than the systems without the acids (Fe^{2+} and Fe^{3+}) (Table 1). In presence of H_2O_2, even at neutral pH, more available iron in the system makes the Fenton process more efficient and consequently induces a faster E. coli inactivation.

(ii) The photo-activity of the formed ferric organo-complexes promotes the formation of extra oxidative species ($O_2^{\bullet-}$/$HO_2^{\bullet}$, $HO^{\bullet}$ and $R^{\bullet}$) and Fe^{2+} through a ligand-to-metal charge-transfer (LMCT) [15]. Indeed, the absorption spectra (SM 6–SM 9) show an increment of the light absorption above 400nm in the presence of the organic acids regarding the system with sole Fe^{3+}. Under sunlight irradiation, the Fe^{3+}-organo complexes are not only able to renew the Fe^{2+} in the system by LMCT, but also could produce more disinfecting species from the generated oxidized ligand complexes (L^{+}) via their reaction with O_2.

(iii) Complementary experiments in absence of H_2O_2 revealed higher inactivation in the Fe^{3+} system than Fe^{3+}:CA; TA; AA; CfA systems (SM 2–SM 5). This means that the produced ROS by

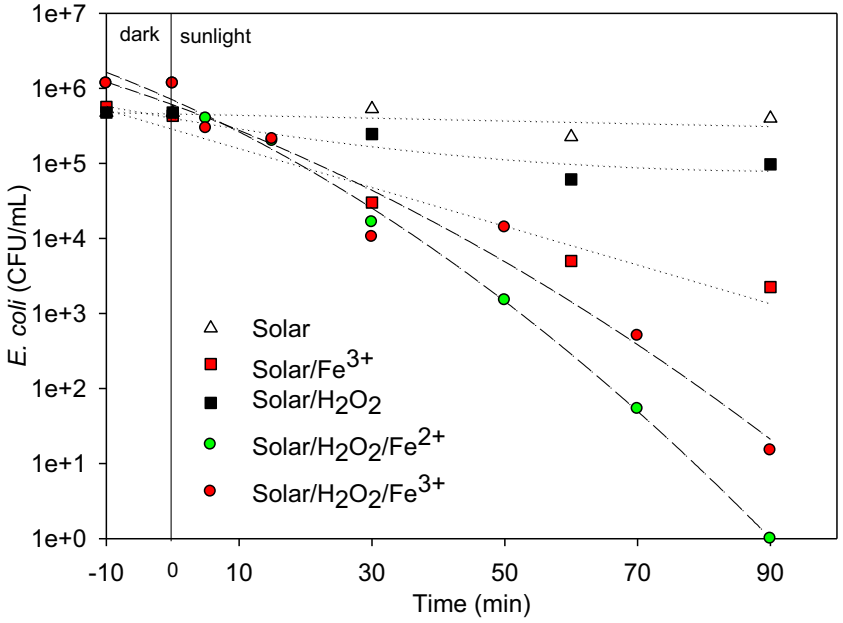

Fig. 1. *E. coli* inactivation by the solar photo-Fenton (Solar/H_2O_2/Fe^{2+} and Solar/H_2O_2/Fe^{3+}) and its process constituents: solar, Solar/Fe^{3+}, Solar/H_2O_2. Initial conditions of the Fenton reactants addition: [$Fe^{2+,3+}$] = 5 ppm; [H_2O_2] = 25 ppm; 600 W/m^2.

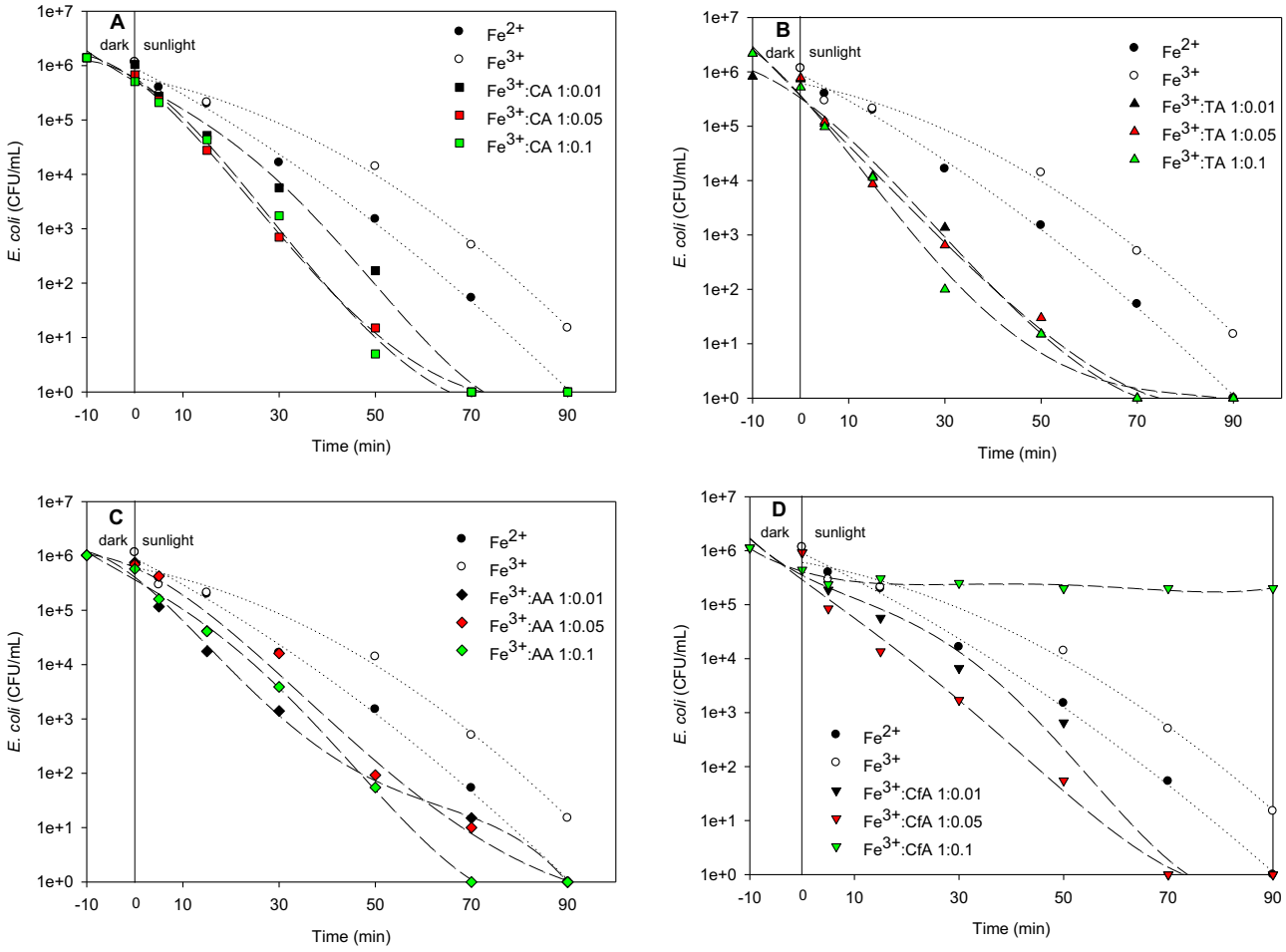

Fig. 2. *E. coli* inactivation by the solar photo-Fenton process driven by Fe^{2+} and Fe^{3+} alone and in the presence of organic acids Fe^{3+}:CA; TA; AA; CfA. Three molar ratios where tested regarding the fixed added iron amounts (1:0.1; 1:0.05; 1:0.01). A) citric acid (CA); B) tartaric acid (TA); C) ascorbic acid (AA); D) caffeic acid (CfA). Initial conditions of the Fenton reactants addition [$Fe^{2+,3+}$] = 5 ppm; [H_2O_2] = 25 ppm; 600 W/m^2.

Table 1
Initial and final total dissolved iron concentration during the *E. coli* inactivation by solar photo-Fenton in the presence of citric, tartaric, ascorbic and caffeic acid at different molar ratios. [$Fe^{2+,3+}$] = 5 ppm; [H_2O_2] = 25 ppm; 600 W/m^2.

System	Initial (ppm)	Final (ppm)
Fe^{2+}	0.35	0.22
Fe^{3+}	0.29	0.14
Fe^{3+}:CA 1:0.01	0.54	0.49
Fe^{3+}:CA 1:0.05	0.61	0.58
Fe^{3+}:CA 1:0.1	0.76	0.56
Fe^{3+}:TA 1:0.01	0.53	0.39
Fe^{3+}:TA 1:0.05	0.55	0.43
Fe^{3+}:TA 1:0.1	0.61	0.46
Fe^{3+}:AA 1:0.01	0.68	0.65
Fe^{3+}:AA 1:0.05	0.81	0.70
Fe^{3+}:AA 1:0.1	0.93	0.70
Fe^{3+}:CfA 1:0.01	0.67	0.52
Fe^{3+}:CfA 1:0.05	0.98	0.57
Fe^{3+}:CfA 1:0.1	0.57	0.30

Fe^{3+}-DOM under solar illumination have (in these conditions) two targets: the bacteria and the OAs.

From points i) to iii), we can suggest that the enhancement of the *E. coli* inactivation by the solar photo-Fenton process with the addition of CA, TA, AA and CfA could be mainly attributed to the Fenton reaction enhanced by the effective regeneration of Fe^{2+} via an LMCT occurring in the Fe^{3+}-OAs complexes.

Previous investigations showed that CA increases the generation of HO• radicals during the Fenton reaction and a fast reduction of Fe^{3+} to Fe^{2+} [52]. This fact is related to the high photo-activity of the complex, attributed to the OH group at the α position, with regard to the carboxylic group. This structural condition contributes to the coordination of the metallic center producing different complexes with variations in the number of ferric ions and citrate ligands [53]. Similarly, the positive effect of tartaric acid can be attributed to the formation of Fe^{3+}-tartrate complex constituted by the combination of ferric ions and the ionized carboxylic groups plus the participation of one or both hydroxyl groups [54]. Consequently, the ferric tartrate complex generate ferrous ion and subsequently promote the Fenton reaction to produce hydroxyl radicals. Then, the enhancement by the CA and TA addition suggests that the LMCT effect (Eqs. (3)-(4)) of the formed ferric-citrate/tartrate complexes has a major contribution in the bacterial inactivation by the reduction of Fe^{3+} to Fe^{2+} and the production of extra HO• radicals.

The addition of AA led to a higher initial dissolved iron quantity than in the presence of the other tested acids. This fact indicates that in the presence of AA, the formation of iron oxides due to the iron precipitation, which have shown poor photo-activity, is less than in the presence of other organic acids. Additionally, the contribution of the possible complexes with AA as ligand can also occur with Fe^{2+} by oxo-bonds with the OH groups of the ring, which combined with dissolved oxygen produce reactive oxygen species including HO• [55]. Furthermore, an additional effect of the ascorbic acid could be associated to the reductive action of this organic molecule which induces the fast formation of ferrous ion from the reduction of ferric ion [35,56]. As a consequence, the iron cycle takes place, and different reactive oxygen species, such as superoxide anion and hydrogen peroxide are generated [57]. The presence of ferrous ion promotes the hydroxyl radicals formation through a homogeneous Fenton reaction (Eq. (1)) and the bacterial inactivation is enhanced. Then, the direct reduction of Fe^{3+} into Fe^{2+} (highly soluble) explains the high concentration of dissolved iron in the beginning of the process.

On the other hand, an enhanced bacterial inactivation was observed when CfA is added in low concentrations (Fe^{3+}:CfA 1:0.01 and 1:0.05). While total inhibition of the process was observed with the highest CfA tested concentration (1:0.1). Contradictory information about the effect of caffeic acid during the Fenton reaction is reported where CfA could inhibit or enhance the hydroxyl radical formation [36,58], but also CfA is known to have antioxidant properties [59].

In principle, the possible formation of ferric complexes with caffeic acid as ligand could be determined by the UV/Vis spectra at different concentrations of CfA (SM 9), where an outstanding absorption of solar radiation (λ > 400 nm) was noticed for all the cases in which Fe^{3+} was mixed with CfA. However, differences were observed according the concentration of CfA. For relatively high concentrations of CfA the spectra are modified with a bathochromic effect and a significant decrease of the intensity suggesting the probable less of photo-active complexes. However, under solar light but in the absence of H_2O_2, the contribution on the *E. coli* inactivation is minimal (SM 5). Moreover, previous investigations have

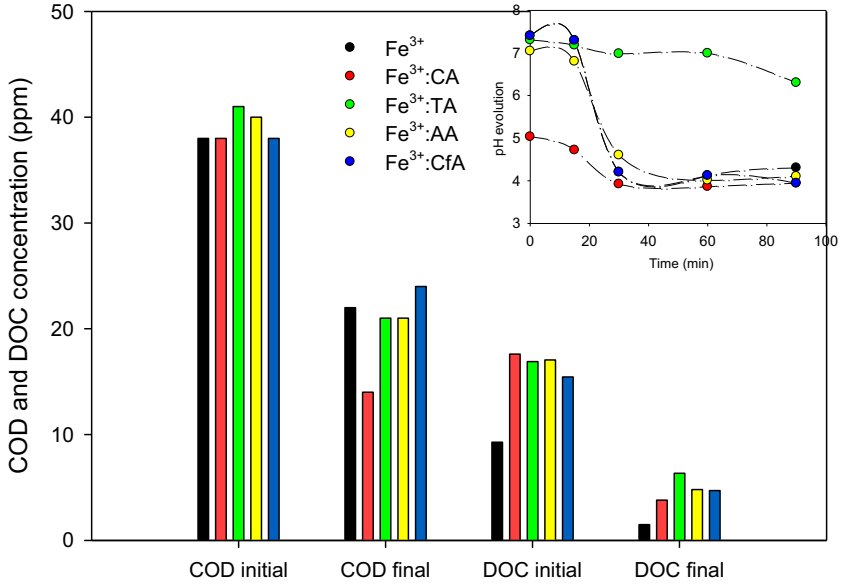

Fig. 3. Initial and final concentration of COD and DOC during *E. coli* inactivation by solar photo-Fenton in the absence and presence of organic acids. Fe^{3+}:CA 1:0.1; Fe^{3+}:TA 1:0.1; Fe^{3+}:AA 1:0.1; Fe^{3+}:CfA 1:0.05. *Inset figure:* pH evolution. Initial conditions of the Fenton reactants addition [Fe^{3+}] = 5 ppm; [H_2O_2] = 25 ppm; 600 W/m^2.

shown that the complexation of both Fe^{3+} and Fe^{2+} with CfA was directly dependent to the $Fe^{2+/3+}$/CfA molar ratios [60], which partially explains the observed differences between our tested molar ratios. Similarly to ascorbic acid, the reductive power of caffeic acid was previously demonstrated when ferric ion was efficiently reduced to ferrous ion in by its action [35], then we suggest that the enhancement of bacterial inactivation by the addition of CfA during the solar photo-Fenton process is determined similarly to AA, by increase of the homogeneous action mode.

3.3. Organic matter fate during the bacteria inactivation in presence of organic acids

As the addition of organic acids into wastewaters raises concerns about increase and the fate of the organic matter, the overall organic charge of the matrix was monitored in the various enhanced photo-Fenton systems. Fig. 3 shows the initial and final values of the chemical oxygen demand (COD) and the dissolved organic carbon (DOC) for the systems with organic acids and compared to the system without any organic acid (Fe^{3+}). For all the systems, both COD and DOC values were significantly reduced along the treatments with slight differences between the processes. The addition of organic acids has not only demonstrated an improved bactericidal activity, but a self-depurative action. However, a mayor elimination of COD was observed when CA was added into the system. Surprisingly, since this difference cannot be attributed to measurement mishaps, we investigate the reasons behind this phenomenon.

During the *E. coli* inactivation tests, we have referred to the effects of pH in the disinfection efficiency, and therefore it is also compared here (inset Fig. 3). Based on the results, in the presence of CA the initial pH is highly acidic (pH ~ 5) compared to the other systems (pH ~ 7). Under such conditions, the Fenton reaction is favored and consequently the global oxidation of the system easily increased, turning into a reduction of the COD.

Moreover, the pH evolution of the systems showed a fast decrease for all the systems (pH 4 at the end of the treatments) except when TA was added, in which the pH drops only to 6. The decrease of the pH is associated to the formation of carboxylic groups from the oxidation of the organic matter presented in the system. Therefore, the slight pH variation using TA suggests a low efficiency of the system to promote the oxidation of the organic matter, which should have a consequence in the iron complexes formation. To support this hypothesis, the total dissolved iron was measured along the treatments in absence of H_2O_2 (SM 10). Dissolved iron increases in all the cases, during the enhanced photo-Fenton treatment. This behavior suggest that during the treatment, one of the first step of the process involves the regeneration of Fe^{2+} by reduction of the Fe^{3+}-complexes, followed by the Fenton reaction where Fe^{3+} is produced. On the other hand, in absence of hydrogen peroxide, the Fenton reaction does not take place and the increment in the dissolved iron could be attributed to the production of ferric complexes during the treatment, with either TA or the organic matter from the wastewater.

Also, considering that the photo-activity of Fe^{3+}:CA and Fe^{3+}:TA to produce Fe^{2+} were the highest (minor difference among them), the large accumulation of iron in the TA system suggests that this acid produce more efficiently ferric complexes along the process. However, the presence of TA easier selectively promotes the *E. coli* inactivation rather than the oxidation of the organic matter. Therefore, in conclusion, since high photo-activity is present, high inactivation potential but low organic matter removal, then we suggest that the radicals produced are probably ROS (O_2^{-}, $^1O_2$) weaker than HO•, oxidized ligand radicals or more possibly, enhanced LMCT between the released iron and the bacterial membrane. These actions withhold the potential of bacterial inactivation rather than oxidation of organic matter.

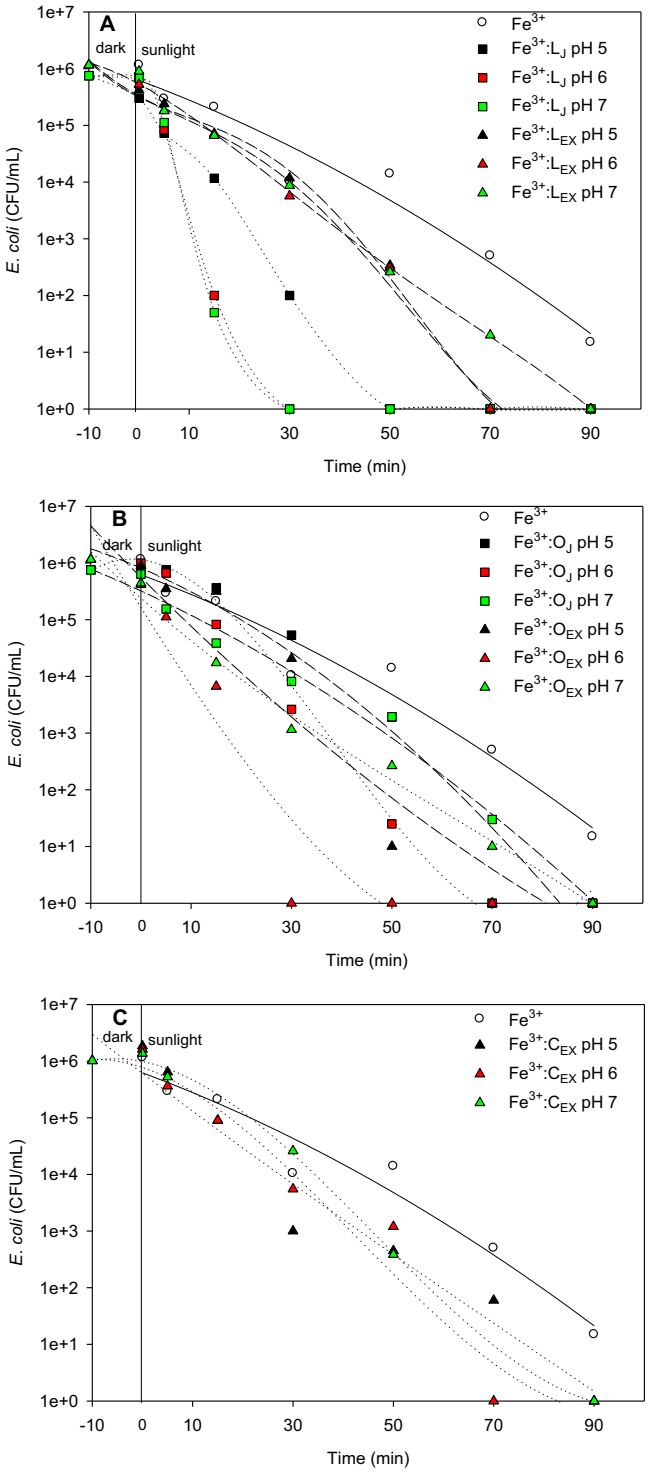

Fig. 4. *E. coli* inactivation by solar photo-Fenton in the presence and absence of natural products at different concentrations represented by pH (5, 6 and 7). A) Lime fruit juice and extract: Fe^{3+}:L_J and Fe^{3+}:L_{EX}; B) Orange fruit juice and extract: Fe^{3+}:O_J and Fe^{3+}:O_{EX}; C) Coffee peel extract: Fe^{3+}:C_{EX}. Initial conditions of the Fenton reactants addition [Fe^{3+}] = 5 ppm; [H_2O_2] = 25 ppm; 600 W/m^2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

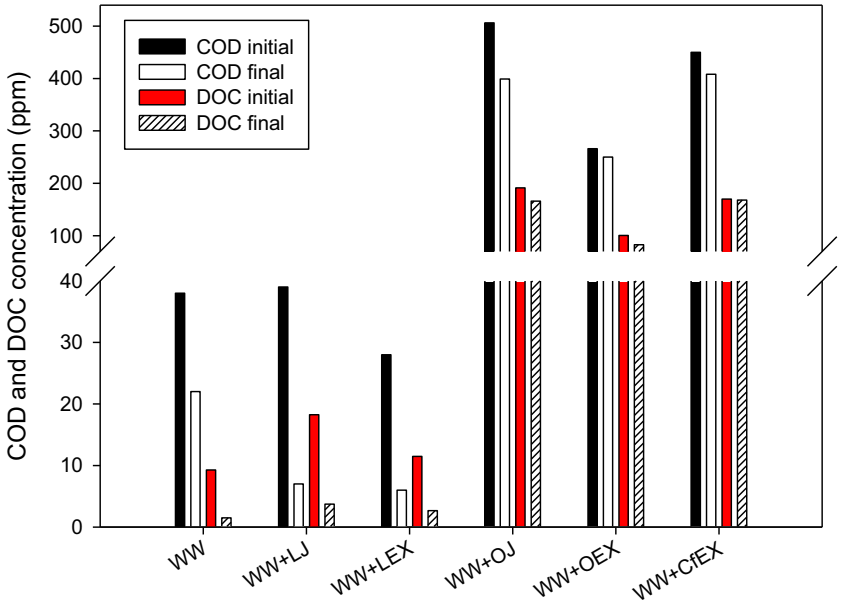

Fig. 5. Initial and final concentration of COD and DOC during *E. coli* inactivation by solar photo-Fenton in the absence and presence of natural products at pH 6. From left to right: WW alone, WW with lime juice, WW with lime extract, WW with orange juice, WW with orange extract and WW with coffee extract. Initial conditions of the Fenton reactants addition [Fe^{3+}] = 5 ppm; [H_2O_2] = 25 ppm; 600 W/m^2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. E. coli *inactivation by the enhanced solar photo-Fenton process with natural sources of organic acids*

Several natural products have the mentioned organic acids as main constitutes in the juices, peels and pulps [28,61,62]. Therefore, the evaluation of natural products as possible sources of the organic acids becomes a new challenge. In this part, two different types of addition were tested: (i) the juices (J) from fresh lime (L) and orange (O) fruits and (ii) an aqueous extraction of the peels (EX) of lime, orange and coffee grain (see Section 2.5). Since the addition of natural products, as juices or extractions, has a direct influence on the pH, the concentration of the natural products was fixed by initial pH, instead of Fe:Acid molar ratio. Fig. 4 shows the effect of L_J, L_{EX}, O_J, O_{EX} and C_{EX} at different concentrations during the *E. coli* inactivation by solar photo-Fenton. Three different addition levels of the natural products were tested corresponding to pH 5, 6 and 7. Notably, the solar photo-Fenton process for bacteria inactivation was enhanced in most of the cases where natural product were added into the systems.

In lime experiments, the addition of either the juice or the extraction accelerated the bacterial inactivation. According to the lime characteristics, both TA and AA are part of the product and could be contributing in the process. However, the observed enhancement should be attributed mainly to citric acid, given its high concentration in both the juice and the peel of the fruit [63]. Furthermore, a remarkable enhancement was observed with L_J at pH 6 and 7 reaching total inactivation after only 30 min of treatment (Fig. 4A). On the other hand, the efficiency was lowered for the case of L_J at pH 5, where inactivation was reached at 50 min of treatment. In order to reach an initial pH of 5, more L_J must be added into the system and the lower inactivation should be attributed to the excess of added organic matter, which competes with bacteria for the oxidizing species, mainly the hydroxyl radicals. In contrast, the lime extractions (L_{EX}) showed a faster inactivation at pH 5 and 6 compared to pH 7. Most possibly, at neutral pH, the amount of added CA is less than a pH 6 and 5, then less ferric complexes are produced and the bacteria inactivation is lowered.

In all the tested systems with the orange juice, an enhanced bacterial inactivation was observed, compared to the photo-Fenton system with Fe^{3+} and no additive (Fig. 4B). Based on the orange composition, the effectiveness of these systems could be attributed to the presence of CA and TA, but primarily to AA ending up into a synergistic mixture. A notable acceleration of the process was obtained with O_{EX} at pH 6 reaching total inactivation of *E. coli* after only 30 min of treatment. These results are attributed to the separation of the organic acids, mainly AA, by the aqueous extraction performed. The extracted acids from the peel are, similarly to the lime experiments, present with reduced competition of extra organic matter presented in the fruit juice.

Finally, the aqueous extraction of the coffee peel (C_{EX}) was also investigated (Fig. 4C). Inactivation of *E. coli* by the addition of coffee extraction can be noticed under all the tested concentrations but less efficient than the lime and orange systems. The best efficiency was observed for the C_{EX} pH 6 system reaching total inactivation after 70 min of treatment while for the lime and the orange experiments happened after 30 min. As previously described, the CfA efficiency during the solar photo-Fenton process is a both function of the amount of acid added into the system and the Fe:CfA ratio. Hence, the observed differences of the efficiency are a result of the initial pH regulation by C_{EX} addition.

In order to explain and generalize the effect of the natural additives on the enhancement of the photo-Fenton action, some tendencies have been observed and summarized as follows:

i) Importance of the Organic Acid/Organic Matter ratio (OA/OM)

During the lime experiments, the differences between the extractions' and the juices' inactivation tendencies at different pH are related to the ratio of the CA and the added organic matter (OM). In the L_J, the ratio CA/OM is lower than in the L_{EX} and consequently L_{EX} pH 5 has less competition of the added organic matter than L_J pH 5. This hypothesis can be confirmed by the initial dissolved organic carbon which is almost twice higher for the juice than the extraction (Fig. 5). Similarly in orange related experiments, at O_{EX} pH 6, the possible inhibition of the organic matter is offset and overcome by the positive effects of the organic acids previously described. This pH value represents less DOC in the system compared to the

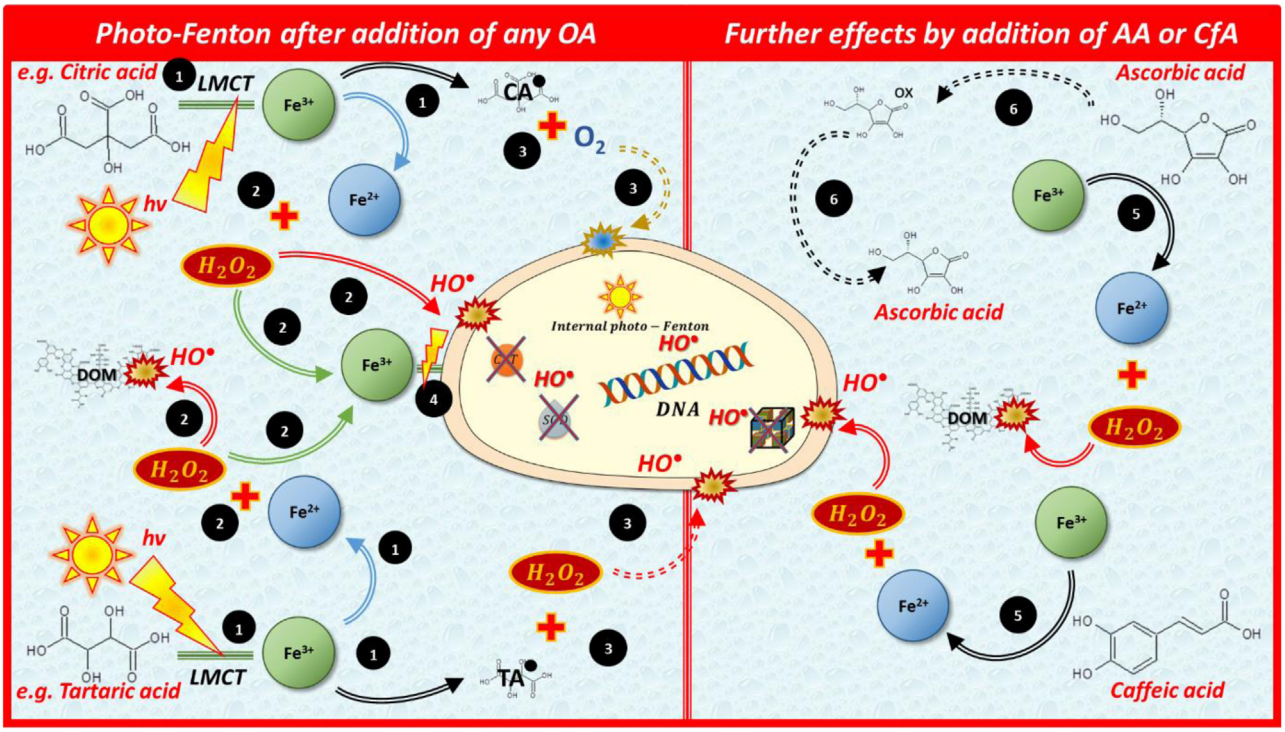

Fig. 6. Proposed mechanism of the enhancement of the photo-Fenton process by the addition of OAs. The left part indicates the modified photo-Fenton process by addition of any among the OAs used in the study, while the right part indicates the extra actions initiated by AA or CfA. The actions 1–6 are explained in detail in Section 3.5.

O_J, which can be evidenced in Fig. 5. Finally, in the C_{EX} experiments, again pH = 6 was found to present the optimal inactivation rates. In conclusion, after regulation-addition of natural products to pH = 7, minimal enhancement is achieved, increasing at pH = 6 and blocked at pH = 5, due to excess OM present in the solution.

ii) Correlation between available iron and organic acid addition

The dissolved iron was determined along the processes, at the corresponding addition of pH 6 of each tested natural product (SM 11). A significant increment of the initial dissolved iron was observed along all the processes, especially when the natural products were added into the systems, similarly to the pure OAs addition. This fact could be attributed to the complexation by the organic matter presented in the media. For the L_J and O_{EX}, the initial iron was almost 5 ppm suggesting a major contribution of the matrix to promote iron complexes. Therefore, the enhancement of these two systems can be attributed to a contribution of the added organic matter that increases the available iron, and which subsequently participates into the Fenton reaction.

Additionally, the bacterial inactivation was also evaluated with Fe^{3+} and natural products under solar radiation without hydrogen peroxide (SM 12). The results showed a favorable inactivation when O_{EX} and especially L_J were part of the systems.

iii) Addition of DOC and COD vs. bacterial inactivation

Fig. 5 shows the COD and DOC initial concentration and after the treatment for the systems with and without the addition of natural products. In all cases, DOC decreased along the treatment, except for the Fe^{3+}:C_{EX} system. Nevertheless, despite the high initial organic matter of the orange juice experiments, the processes achieved bacterial inactivation while merely promoting the oxidation of the organic matter. Therefore, these findings show the possibility of the natural products to act as organic acid sources for the efficient bacterial inactivation by the formation of photo-active ferric complexes. The small pH modification and the biodegradable nature of the additives makes them a potentially attractive solution where these products are available.

3.5. Participation of OAs of natural products in the bacterial inactivation mechanism: enhanced solar photo-Fenton process

According to the experimental results and the mechanisms described, in Fig. 6, we illustrate the enhanced photo-Fenton process and summarize the mechanisms implicated by the addition of OAs. The supporting references will not be repeated here. Actions 1–4 are predominant in all acids while 5 and 6 appear mostly in AA and CfA.

1) The addition of OAs in WW enhances the complexation of iron and subsequently the photo-activity of the organo-complexes. This leads to enhanced LMCT and reduction of Fe^{3+} to Fe^{2+}. Also, a ligand radical is produced.
2) The newly produced Fe^{2+} participates in the Fenton reaction and accelerates the production of HO• radicals, while oxidized in Fe^{3+}. The HO• radical non-selectively attack bacterial cells and DOM in the solution.
3) The ligand radicals can react with oxygen or H_2O_2 in water, leading to the generation of mild oxidants, such as superoxide radical or singlet oxygen, or HO• radical, respectively.
4) The iron formed can complex with the bacterial membrane and thereby facilitate LMCT, damaging the cell wall and replenishing the Fe^{2+} in the bulk.
5) Direct reduction of Fe^{3+} to Fe^{2+} can be facilitated by AA and CfA. As a result, the homogeneous Fenton action is promoted.
6) The oxidized ligand, as well as the OA itself, can react with the H_2O_2, either returning to normal state, or consume the oxidant on the expense of the homogeneous Fenton process. The OAs can further complex Fe^{3+} and sustain the LMCT reactions.

However, although different possible action modes deriving from the illuminated iron-OAs complexes have been identified as related to the specific OA structure, the remarkable observed enhancement of E.coli inactivation rates in wastewater were

similar, with small differences, among the various OAs. This means that in our conditions the driving force of the four tested systems was the supply of significant amounts of soluble iron, which fed and enhanced the (photo)Fenton process. In our case, the ROS generated from oxidized ligands in contact with oxygen, as well as the direct reduction by AA and CfA seems to play a secondary role on bacterial inactivation rates.

4. Conclusions

This investigation proved the enhancement of *E. coli* inactivation in wastewater using citric, ascorbic, tartaric and caffeic acid into the system, to enhance the solar photo-Fenton process. Different factors are involved, as a function of the added organic acids. For all cases, a major contribution on the process was identified and associated to the formation of ferric complexes with photoactive activity. This characteristic leads to a photo-assisted LMCT that causes enhanced reduction of Fe^{3+} to Fe^{2+} which, in turn, easily undergoes into the Fenton reaction producing hydroxyl radicals. However, in the presence of AA and CfA, there is also a significant contribution of a direct reduction of ferric ion given their antioxidant properties.

The evaluation of natural products highly concentrated with the aforementioned acids showed an enhanced effect on the solar photo-Fenton efficiency. The favorable effects are predominant, leading to effective bacterial inactivation, despite the expected inhibition by the added organic matter in the treated water. These findings indicate a new, green, alternative way to efficiently enhance the solar photo-Fenton process for the *E. coli* inactivation in wastewater under mild, natural conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apcatb.2016.12.021.

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